

FRACTIONATION OF THE NUCLEAR MATRIX

I. Partial Separation into Matrix Protein Fibrils and a Residual Ribonucleoprotein Fraction

RONALD BEREZNEY

From the Division of Cell and Molecular Biology, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York 14260

ABSTRACT

Isolated rat liver nuclear matrices have been partially separated by means of mild sonication into a matrix protein (matricin) fraction and a residual ribonucleoprotein (RNP) fraction. The initial matricin fraction is composed largely of protein (91.1%) but also contains significant amounts of DNA (8.4%). Reconstruction experiments indicate that this DNA is not the result of the artifactual binding of DNA to the matrix during the extraction procedures. Subsequent treatment with DNase I results in purified matricin composed of >99.5% protein. SDS acrylamide gel electrophoresis of the matrix protein fibrils reveals only three bands: the primary matrix polypeptides of 62,000, 66,000, and 70,000 daltons. Electron microscopy demonstrates a diffuse reticulum with fibrils as thin as 30–50 Å and the presence of 80–100-Å globular structures. The residual RNP fraction is composed largely of protein (80.1%) and RNA (19.5%), with only traces of DNA (1.1%). Over 98% of the total matrix-associated RNA is recovered in this fraction. SDS acrylamide gel electrophoresis indicates an enrichment in both low and high molecular weight secondary matrix polypeptides, although the 60,000–70,000-dalton polypeptides are present in significant amounts as well. Ultrastructural analysis of the residual RNP fraction reveals distinct electron-dense-staining matrix particles (150–350 Å) attached to a fibrous matricin network.

By suitable extraction procedures, a proteinaceous nucleoskeletal matrix has been isolated from a variety of eukaryotic nuclei (4, 6, 7, 9, 10, 19, 28, 30, 31, 54). The close similarity of the isolated matrix structures to *in situ* observed nuclear structures suggests that the isolated matrix is not an artifact of preparation (4, 6, 10). This is further supported by the cytological identification of an overall nuclear matrix structure in both fixed and unfixed whole cells (15, 26, 51). Moreover, structural alterations induced in the *in situ* matrix structure by actinomycin D are maintained in the

corresponding isolated nuclear matrices (27).

Although considerable differences may exist in the overall polypeptide composition of nuclear matrices prepared from different tissues and cell lines (4), polypeptides in the range of 60,000–70,000 daltons generally predominate. The presence of polypeptides of similar molecular weight as major constituents of a variety of isolated nuclear fractions (1, 4, 10, 17, 32, 48) suggests a fundamental structural role for these nonhistone proteins. In this regard, a number of *in situ* observations have indicated proteinaceous fibrous struc-

tures, generally 30–100 Å in width, associated with or in close proximity to a variety of intranuclear structures including interchromatinic and perichromatinic fibers and granules (12, 42, 50), nuclear pore complexes (34, 36, 38, 53), nucleoli (46, 47), and *in situ* observed matrix structures (15, 26). Protein fibrils of similar dimensions and staining characteristics are a major structural component of the isolated nuclear matrix. These matrix protein fibrils have been termed “matrixin” or “matricin” by Comings and Okada (19), who further suggest that they are composed of one or more of the 60,000–70,000-dalton matrix polypeptides.

In this communication, I report the partial resolution of the matrix structure. Matricin is isolated. It is composed of all three 60,000–70,000-dalton matrix polypeptide bands in association with approximately two-thirds of the total matrix-associated DNA. This DNA is entirely removed by DNase digestion to yield purified matricin. A residual ribonucleoprotein (RNP) fraction contains essentially all the matrix-bound RNA and the secondary matrix polypeptides and is enriched in matrix particle structures.

MATERIALS AND METHODS

Preparation of Nuclei and Nuclear Matrix

Highly purified liver nuclei were isolated from male rats (Sprague-Dawley, 200–250 g, purchased from Blue Spruce Farms, Altmont, N. Y.), and nuclear matrices were prepared from isolated nuclei by a previously reported procedure (10). All steps were performed at 0°C, with centrifugation at 1,000 *g* for 15 min in a Sorvall HS-4 rotor (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.). Nuclei were resuspended to 1 mg DNA/ml in STM buffer (0.25 M sucrose, 20 mM Tris, pH 7.4, 5 mM MgCl₂) and digested with 5 µg of DNase I (Worthington Biochemical Corp., Freehold, N. J.) per milliliter for 10 min. In some experiments, nuclei were endogenously digested (14 h, 5°C) instead of being treated with DNase I, or 1 mM phenylmethylsulfonyl fluoride was added to the DNase I stock solution (500 µg/ml). After centrifugation, the DNase-treated or endogenously digested nuclei were extracted with low-magnesium (LM) buffer (0.2 mM MgCl₂, 10 mM Tris, pH 7.4), followed by three consecutive extractions of the nuclear pellet with high-salt buffer (2 M NaCl, 0.2 mM MgCl₂, 10 mM Tris, pH 7.4), one extraction with 1% Triton X-100 in LM buffer, and two final washes in LM buffer. Nuclear matrices prepared in this manner have substantial amounts of tightly bound RNA and DNA. In some experiments, 1 mM phenylmethylsulfonyl fluoride and 0.1 mM tetrathionate were added to the matrix isolation solutions (5).

Matrix Fractionation

Nuclear matrices in LM buffer (1 mg protein/ml) were sonicated with three 30-s bursts at ~60 W with a Branson sonicator model W-140 (Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) while the temperature was maintained below 4°C. The matricin fraction was pelleted by centrifugation of the matrix sonicate at

5,000 *g* for 15 min in the HS-4 rotor. The RNP fraction was obtained by centrifugation of the corresponding supernate at 100,000 *g* for 16 h in a Beckman 50 Ti rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Matricin was purified from the matricin fraction by digestion with 50 µg of DNase per milligram of matrixin protein at 0°C for 60 min in 5 mM MgCl₂, 10 mM Tris, pH 7.4, followed by centrifugation of the matricin at 5,000 *g* for 15 min and two washes in LM buffer.

Analysis of Nuclei and Nuclear

Matrix Fractions

Thin-sectioning electron microscopy, SDS acrylamide gel electrophoresis, and protein, RNA, and DNA determinations are detailed elsewhere (10). Histones were analyzed by extraction with 0.25 N HCl followed by electrophoresis according to Panyim and Chalkley (44).

Isolation of Labeled DNA

Labeled DNA for *in vitro* binding experiments was isolated from 24-h regenerating rat livers. Livers were removed 1 h after injection of 200 µCi of [³H-methyl]thymidine (55 mCi/mmol; New England Nuclear, Boston, Mass.) into the hepatic portal vein, and nuclei were isolated. Endogenously digested nuclei (14–16 h, 5°C; in 0.25 M sucrose, 5 mM MgCl₂, 20 mM Tris, pH 7.4) were resuspended to a concentration of 10 mg DNA/ml and mixed with 50 vol of lysis solution (2% SDS, 7 M urea [Ultrapure, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.], 0.35 M NaCl, 1 mM EDTA, 10 mM Tris, pH 8) containing 100 µg/ml of proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.; predigested at 37°C for 30 min) and incubated at 37°C for 1 h. The aqueous suspension was then extracted repeatedly with a 1:1 mixture of phenol-chloroform containing 1% (vol/vol) isoamyl alcohol and 0.1% (wt/vol) 8-hydroxyquinoline until the aqueous-phenol interphase was clear. After ethanol precipitation of the aqueous phase, the DNA was suspended in 5 mM MgCl₂, 10 mM Tris, pH 7.4, and digested with 200 µg/ml of pancreatic RNase (Worthington Biochemical Corp.; predigested at 100°C for 10 min), followed by proteinase K digestion and phenol-chloroform extraction as described above. The isolated DNA sediments between 4S and 17S on alkaline sucrose gradients, with an average of 7.7S. This corresponds to a single-strand size of ~0.8 kilobase.

RESULTS

Fractionated Nuclear Matrices

Nuclear matrices isolated from rat liver were fractionated into matricin and RNP fractions as described in Materials and Methods. Nuclear matrices were exposed to mild sonication, and the matricin network was isolated by low-speed centrifugation (5,000 *g*, 15 min). The residual RNP fraction was obtained by centrifugation of the 5,000-*g* supernate at 100,000 *g* for 16 h. Approximately 97% of the total matrix proteins are sedimented under these conditions. The matricin fraction contained 26% of the total matrix protein, 70% of the total matrix-associated DNA, and <1% of the total matrix RNA (Table I), and was com-

TABLE I
Macromolecular Composition of Fractionated Nuclear Matrices

Fraction	Protein*	RNA*	DNA*
	mg	mg	mg
Total nuclei	162.0 ± 9.4	7.14 ± 0.5	48.2 ± 4.7
Nuclear matrix	15.7 ± 2.1	2.75 ± 0.7	0.52 ± 0.07
Matricin fraction	4.1 ± 0.8	0.02 ± 0.01	0.38 ± 0.05
Matricin fibrils‡	4.0 ± 0.6	<0.01	<0.01
RNP fraction	11.2 ± 1.9	2.72 ± 0.80	0.16 ± 0.02

* Values are based on 50 g wet wt of rat liver and represent the mean ± SD for three preparations.

‡ Matricin fibrils are purified from the matricin fraction by digestion with DNase I (50 µg/mg matricin protein) at 0°C for 60 min (see Materials and Methods).

posed of 91.1% protein, 8.4% DNA, and <0.5% RNA (assuming percent protein + percent DNA + percent RNA = 100%). The matricin fraction was then digested with DNase I (50 µg DNase per milligram of matricin protein) at 0°C for 60 min. The resulting purified matricin was composed of >99.5% protein (Table I). The addition of phenylmethylsulfonyl fluoride (1 mM) to the DNase I stock solution (1 mg/ml) immediately before digestion had no detectable effect on the composition, morphology, or polypeptide profile of the final protein fibrils.

The DNA tightly associated with matricin before DNase treatment is not a result of nonspecific trapping or cosedimentation of the DNA with the matricin. DNA prepared after endogenous digestion of the nuclei binds in only trace amounts to isolated nuclear matrices or nuclei during the various stages of matrix isolation (Table II). The amount of bound, labeled DNA, which is barely above background radiation (20–25 cpm), was not increased by a 10-fold increase in either the amount of labeled DNA or the amount of nuclear fraction. This indicates that the trace amount of labeled DNA that binds is not significant. Moreover, the tightly bound matrix DNA fragments sediment in the range of 4–17S on alkaline sucrose gradients (R. Berezney, unpublished observations) and would, therefore, remain at the top of the centrifuge tube after sedimentation of the matricin fraction (see Materials and Methods). Other reconstruction experiments by Miller et al. (41) suggest that hnRNA associated with the nuclear matrix is also not an in vitro artifact of preparation.

The residual RNP fraction contains the bulk of the total matrix protein (71%) and RNA (99%), with a lesser amount of the total matrix-associated DNA (~30%), and is composed of 79.5% protein, 19.3% RNA, and 1.1% DNA.

TABLE II
In Vitro Binding of Labeled DNA to Nuclei During Nuclei and Matrix Isolation

DNA addition*	Total cpm bound	Percent of total cpm
Whole tissue	14	0.3
Isolated nuclei	10	0.2
During low-magnesium extraction	9	0.2
During high-salt extraction	14	0.3
Final nuclear matrix	12	0.2

* DNA was isolated from regenerating liver pulsed in vivo for 60 min as described in Materials and Methods. To approximate conditions present during nuclear and matrix isolation, the DNA was isolated after endogenous digestion of nuclei (14 h, 5°C) in STM buffer. 200 µg of labeled DNA was incubated with the nuclei (2 mg DNA) for 15 min at 0°C at different stages of the nuclei or matrix isolation. The corresponding nuclear matrices were then isolated, and the bound radioactive DNA was measured. This measures DNA that binds in vitro and is subsequently resistant to extraction with 2 M NaCl, as is the case with the tightly bound matrix DNA (3, 7, 8). Specific activity of the 60-min pulsed DNA was 25,000 cpm/mg DNA. No increase in counts per minute bound was obtained by increasing either the amount of total nuclei or the amount of labeled DNA 10-fold.

Ultrastructure of the Purified Matricin and RNP Particles

The structure of the isolated rat liver nuclear matrix is shown in Fig. 1. The predominant component of the matrix is an elaborate fibrous internal matrix, which also contains electron-dense-staining matrix particles. Although residual nucleolar structures are often present (not shown in Fig. 1), they appear to represent, along with the residual nuclear envelope, relatively minor structural components. At higher magnification (Fig.

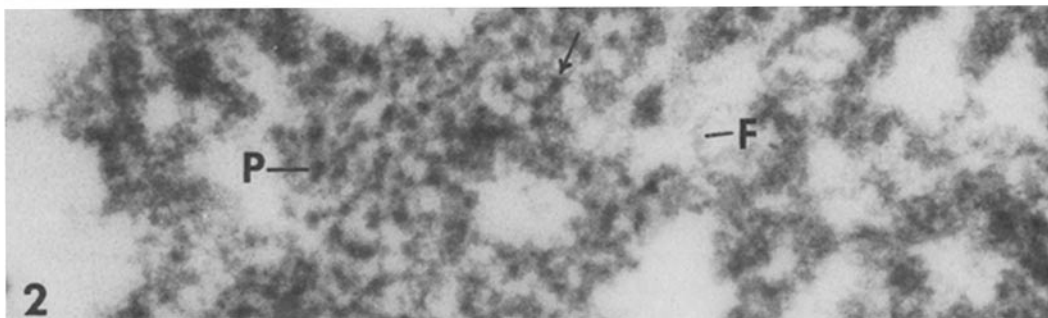
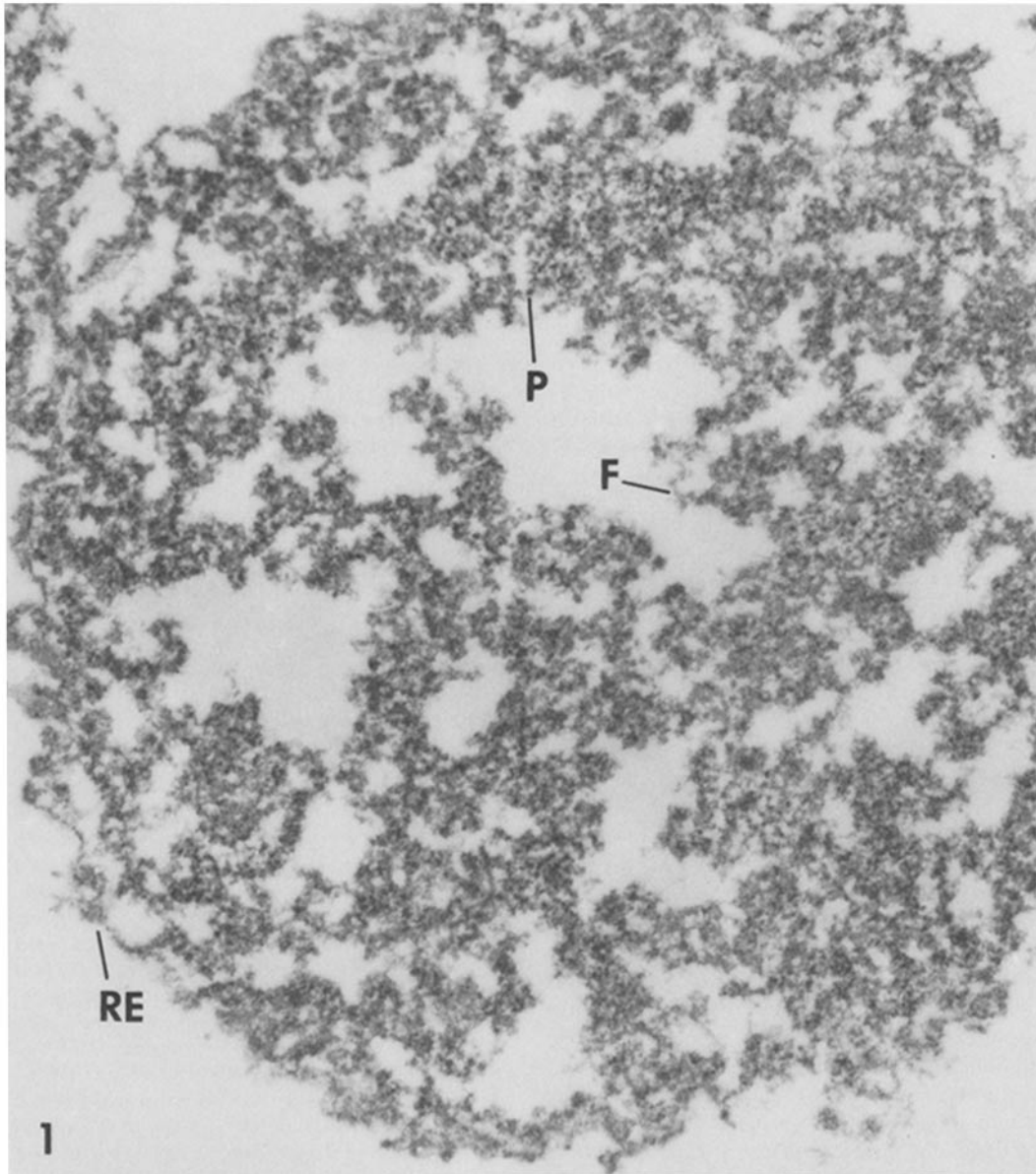


FIGURE 1 Thin sections of rat liver nuclear matrix. Nuclear matrices were isolated as described in Materials and Methods. The predominant structure is an elaborate internal matrix that consists of a diffuse reticulum of fibrils (*F*) and associated matrix particles (*P*). *RE* denotes the surrounding residual nuclear envelope layer. $\times 40,000$.

FIGURE 2 Higher magnification of the nuclear matrix. Electron-dense-staining matrix particles (*P*) are attached to a less electron-dense, diffuse reticulum of fibrils (*F*). The arrow points to a characteristic chainlike array of matrix particles that appear to be interlinked by matrix fibrils. $\times 100,000$.

2) the particulate (*P*) and fibrous (*F*) structures of the internal matrix are more clearly observed. The fibrils are packed into a diffuse matrix. Individual fibrils as thin as ~30–50 Å are observed and may represent unit matrix. The matrix particles are very electron-dense-staining, ellipsoidal structures, with dimensions of 150–350 Å, and appear to be attached to the fibrous matrix. Apparent matrix often appears to interconnect matrix particles (arrow, Fig. 2).

The purified matrix (after DNase treatment) consist of a reticulum of protein fibrils (Fig. 3). 30–50-Å fibrils are readily observed. The bulk of the fibrous structures, however, are much thicker (70–120 Å), which might be the result of either the coiling of individual unit fibrils or the self-association of two or more fibrils. Globular structures of ~80–100 Å (arrow Fig. 3) are commonly observed and may correspond to a highly coiled state of the protein fibrils.

The residual RNP fraction is rich in the electron-dense-staining matrix particle structures (Fig. 4). The dimensions of these isolated particles are similar to those in the total internal matrix structure (~150–350 Å). Moreover, a considerable number of fibrous matrix structures are still associated with the particles. These tightly bound fibrils may be involved in the interlinking of the matrix particles into linear arrays (arrows, Fig. 4). Similar interlinked matrix particles were observed in the isolated nuclear matrix (arrow, Fig. 2).

SDS Acrylamide Gel Electrophoresis of the Polypeptides from Matrix and the RNP Fraction

Resolution of the total nuclear matrix proteins on SDS acrylamide gels (Fig. 5) indicates a polypeptide profile similar to that previously reported (7, 10). Although the primary 60,000–70,000-dalton matrix proteins are prominent, the more diverse secondary matrix proteins make up ~60% of the total stained protein in the polypeptide profile (Table III). Purified matrix consists largely of the three primary matrix polypeptide bands of 70,000, 66,000, and 62,000 daltons. No other defined polypeptide bands are detected either by visual inspection of the gels or by high-resolution densitometric scanning and subsequent quantitation (Fig. 5 and Table III). In contrast, the residual RNP fraction is concentrated in the secondary matrix polypeptides (Fig. 5), which make up ~80% of the Coomassie Blue-stained protein (Table III).

This includes eight main polypeptides (57,000, 55,000, 50,000, 44,000, 35,000, 30,000, 27,000, and 13,000 daltons) between 57,000 and 13,000 daltons, as well as a large number of high molecular weight secondary matrix polypeptides (>90,000 daltons). The band at 13,000 daltons is apparently not histone because extraction of the total nuclear matrix or RNP fraction with 0.25 N HCl and resolution of the acid-soluble fraction on urea acrylamide gels revealed the absence of defined histones. This is consistent with previously reported results (10).

It is possible that the differential distribution of matrix polypeptides into the purified matrix and the residual RNP fraction is a consequence of the degradation of matrix polypeptides during the fractionation and isolation process. To rule out this possibility, the total recovery of primary and secondary matrix polypeptides in these two fractions was estimated (Table III). The results demonstrate an excellent correlation between the recovery of protein in the primary and secondary matrix polypeptide classes (95–99.0%, Table III) and the recovery of total matrix protein (96.8%, Table I). Moreover, the addition of both serine-specific (1 mM phenylmethylsulfonyl fluoride, reference 23) and sulfhydryl-specific (0.1 mM sodium tetrathionate, reference 39) protease inhibitors at all stages of the nuclear and matrix isolation resulted in a similar distribution of polypeptides into the matrix and RNP fractions. In this case, however, there was an increased proportion of high molecular weight polypeptides in the RNP fraction, which closely corresponded to the increased amount of high molecular weight polypeptides observed in the total nuclear matrices (5). A similar distribution of polypeptides into matrix and the RNP fraction was also found when the initial digestion of isolated nuclei with DNase I was replaced by endogenous digestion of the nuclear DNA for 14–16 h at 5°C (5, 29, 33), or when 1 mM phenylmethylsulfonyl fluoride was added to the DNase I stock solution.

DISCUSSION

Fractionation of the Nuclear Matrix

The nuclear matrix proteins can arbitrarily be divided into two major classes: a class consisting of three primary matrix polypeptide fractions of 62,000, 66,000, and 70,000 daltons and a diverse class of secondary matrix polypeptides. Comings and Okada (19) have suggested that one or more

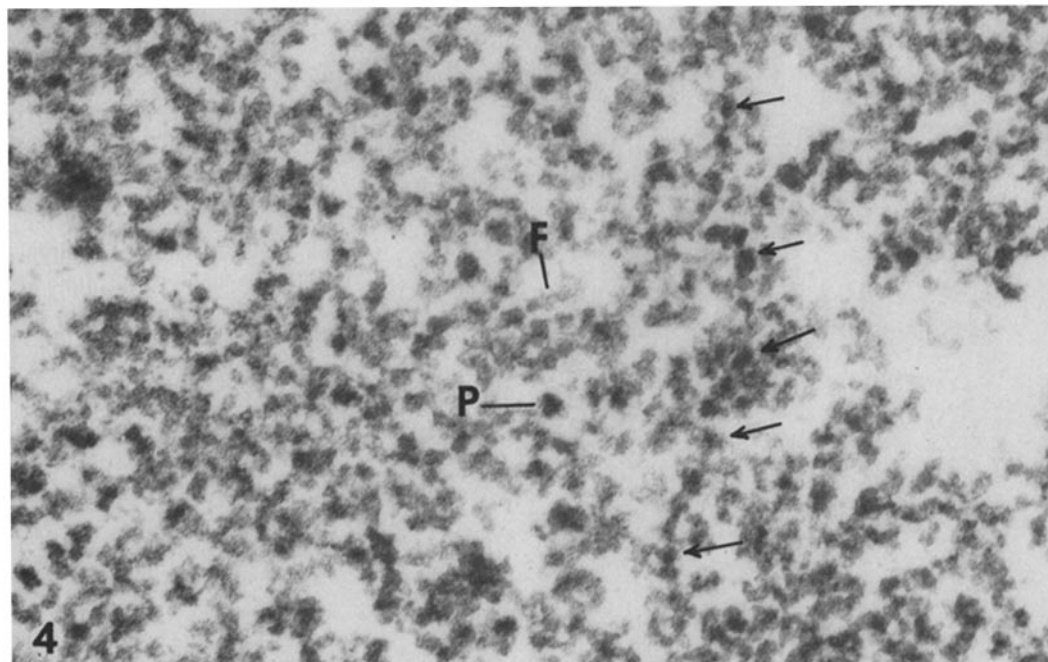
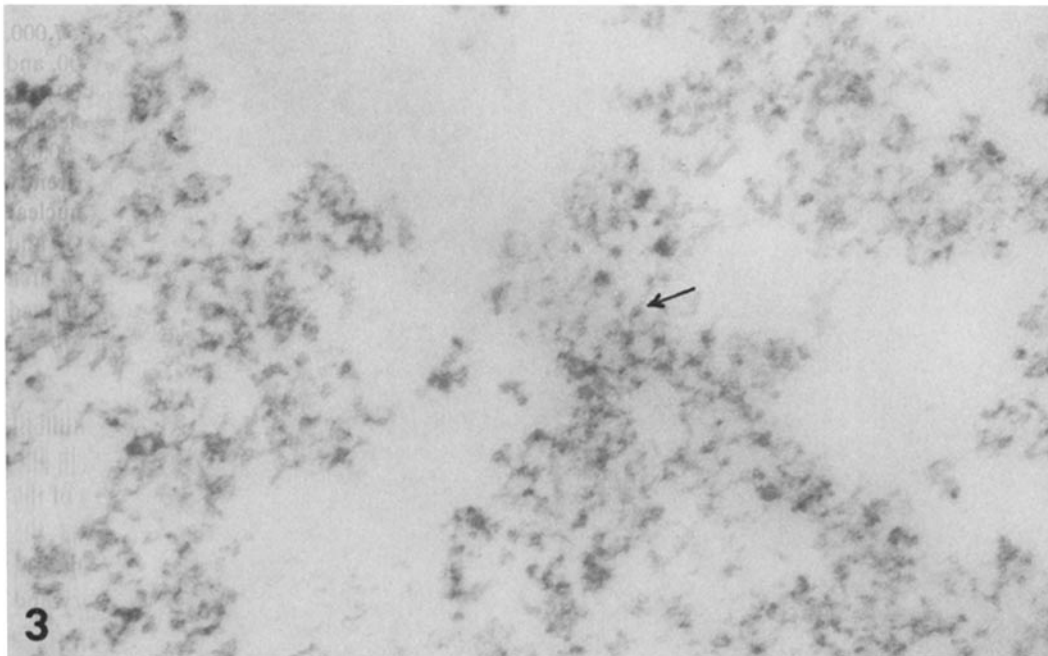


FIGURE 3 Purified matrix. The isolated fibrils resemble structures observed in the intact nuclear matrix (see Figs. 1 and 2) and form a diffuse reticular structure. Fibrils as thin as 30–50 Å are visible. Many globular structures are arranged along the length of the fibrils (arrow). $\times 100,000$.

FIGURE 4 Residual RNP fraction. Although this fraction is rich in matrix particles (*P*), fibrous matrix can also be detected (*F*). Chainlike arrays of particles appear to be interlinked by matrix (arrows). $\times 100,000$.

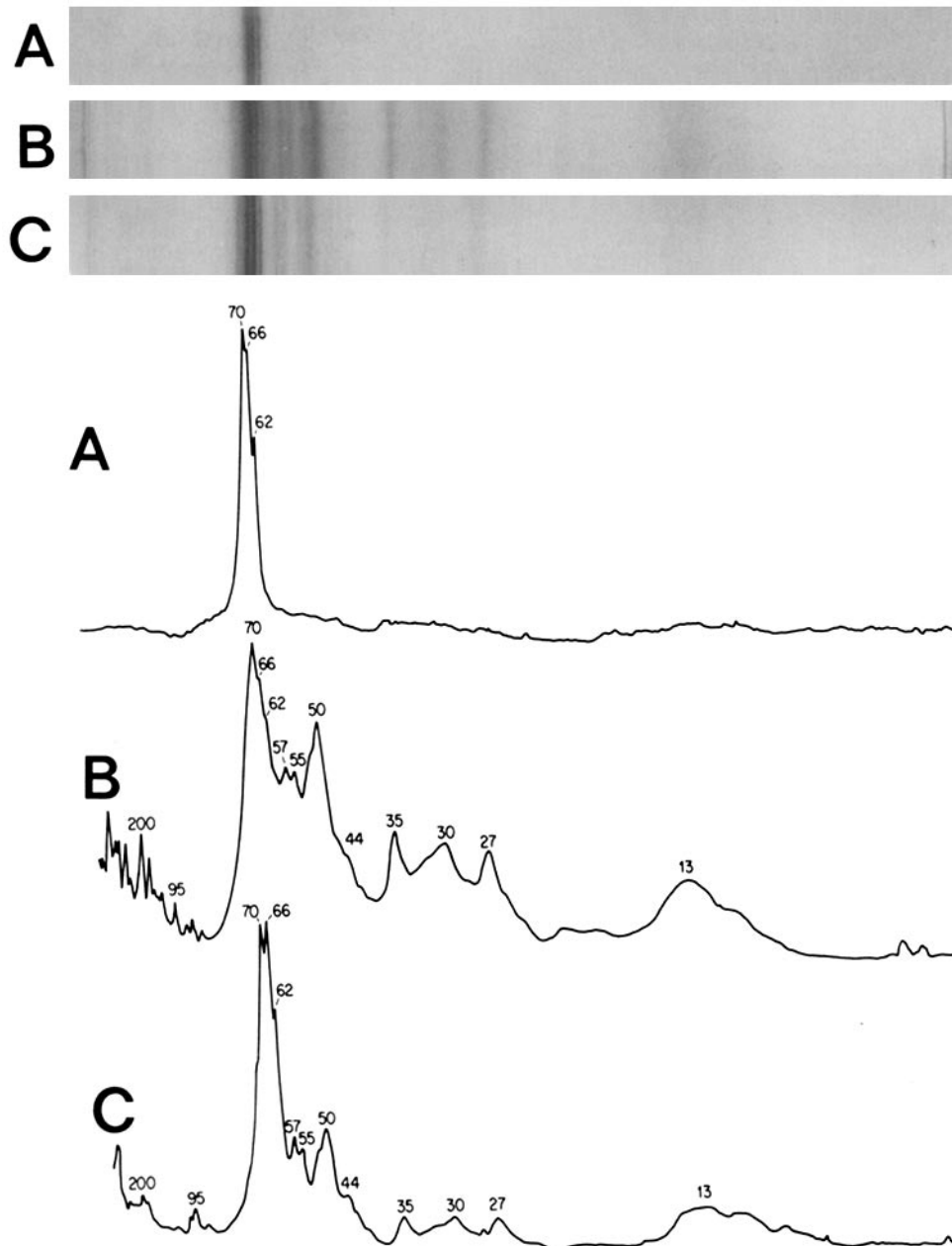


FIGURE 5 SDS acrylamide gel electrophoresis of matrix proteins. The SDS solubilized polypeptides were run on 10% acrylamide gels as previously described (4). Electrophoresis is from left to right. Densitometric tracings are shown below the photographed gels. The numbers above the polypeptide peaks indicate the estimated apparent molecular weights $\times 10^{-3}$. (A) Matrixin, 30 μg protein. (B) Residual RNP fraction, 50 μg protein. (C) Total nuclear matrix, 40 μg protein.

of the primary matrix polypeptides form proteinaceous fibrils (matrixin or matrixin) that are observed both in the isolated nuclear matrix (10, 19) and in the nuclei of intact cells (15, 26). The

secondary matrix polypeptides, however, represent ~60% of the total matrix protein, as determined by densitometry (Table III), and could, therefore, be a major component of the matrixin network.

TABLE III
Densitometric Analysis of Matricin and RNP Polypeptide Profiles

Matrix polypeptide class	Percent of total Coomassie Blue-stained protein			Percent of total matrix polypeptide recovered in the matricin and RNP fractions*
	Total matrix	Matricin fibrils	RNP fractions	
Primary polypeptides (60,000–70,000 daltons)	40.8	100.0	20.1	99.0
High molecular weight secondary polypeptides (>90,000 daltons)	10.1	ND	13.6	95.1
Low molecular weight secondary polypeptides (13,000–57,000 daltons)	49.1	ND	66.3	96.3

* A theoretical recovery of 97.5% is calculated as the percent of total matrix protein recovered in the matricin and RNP fractions based on the data of Table I. Although this represents an "average" recovery of the matrix polypeptide population, it is a valid approximation because >98% of the matrix polypeptides are solubilized by the SDS electrophoresis procedure. The experimental recovery is calculated by multiplying the percent of Coomassie Blue stain in each polypeptide molecular weight class by the percent of total matrix protein recovered in the matricin and RNP fractions, respectively (26.1% for matricin and 71.3% for RNP; see Table I). The sum of these two values is then divided by the percent Coomassie Blue stain in the corresponding total matrix polypeptide class. ND, not detected.

To resolve this question, a major portion of the matricin was purified from isolated nuclear matrices by a brief sonication procedure, followed by DNase treatment. The isolated matricin has a characteristic diffuse structure, with individual fibrils as thin as 30–50 Å. The presence of globular, beadlike structures (80–100 Å) along the fibrils may reflect an ability to coil into various configurations. SDS acrylamide gel electrophoresis indicates that matricin consists of the three primary matrix polypeptide bands, with no detectable secondary polypeptides. Moreover, the secondary matrix polypeptides were quantitatively recovered in a residual RNP fraction that contained electron-dense-staining matrix particles of ~150–350 Å, suggesting a possible structural compartmentalization of the primary and secondary matrix proteins. The primary 60,000–70,000-dalton matrix polypeptides may be common to a variety of nuclear structures in the form of a fibrous matricin network. The secondary matrix polypeptides, however, may have a role in the structural specificity of individual nuclear structures. Consistent with this interpretation, both the residual nuclear envelope and the residual nucleolar components of the matrix contain 60,000–70,000-dalton matrix polypeptides but are considerably different in their secondary matrix polypeptides (9, 10).

Approximately two-thirds of the total 60,000–70,000-dalton matrix polypeptides are recovered in the isolated matricin, as estimated from protein (Table I) and densitometric (Table III) measurements. Most of the remaining primary matrix poly-

peptides are found in the RNP fraction. This is consistent with electron microscope observations demonstrating the persistence of a fibrous matrix to which matrix particles appear to be anchored (Fig. 4). It should be noted that the exact structural origins of the matricin and RNP fractions have not been defined in this study. Although the electron microscope observations strongly suggest that these two fractions are largely derived from the predominate internal matrix structure, the contribution of the residual lamina layer and nucleoli to these fractions cannot be precisely evaluated. To clarify this point, it will be necessary to effectively separate the internal matrix from the residual nucleolar and lamina fractions. Steps have been taken in this direction, but the results are still preliminary (11).

Matrix vs. Lamina Proteins

Recent immunological localization studies (22, 25, 37) suggest that the three primary matrix polypeptides that comprise matricin are actually lamina proteins that are exclusively localized in the nuclear envelope. These results, however, should be interpreted with caution. Although the immunocytochemical results clearly indicate the presence of the 60,000–70,000-dalton polypeptides in the nuclear envelope of isolated nuclei, the absence of reaction does not prove that these polypeptides are not also present in the nuclear interior. The antigenic sites of the internally localized matrix proteins may be nonreactive with the antibodies as a result of either steric or conformational fac-

tors. The organization of the primary matrix polypeptides into a complex, three-dimensional matrix network that is demonstrated in the present study could provide the structural basis for non-reactivity. Moreover, the previous studies were performed with antibodies prepared from SDS-solubilized polypeptides. Such antibodies, however, may be unreactive with the corresponding native proteins under certain conditions (40, 49).

It must be emphasized that approximate quantitation of the 60,000–70,000-dalton proteins, using reported densitometric measurements and total protein recovery values (21), demonstrates a 4–5-fold higher content of these proteins in the total matrix as compared with the lamina fraction. In addition, there is no apparent enrichment of these proteins in isolated lamina vs. matrix fractions. These comparisons suggest that the lamina 60,000–70,000-dalton proteins are quantitatively a subclass of the total matrix 60,000–70,000-dalton proteins. This is also anticipated from morphological considerations because the lamina is only the peripheral layer of the matrix structure (Fig. 1). The crucial question, however, is whether the lamina 60,000–70,000-dalton proteins are an antigenically distinct subclass of the total matrix 60,000–70,000-dalton proteins. Although this remains to be resolved, Peters and Comings (45) have demonstrated that the 60,000–70,000-dalton polypeptides of nuclear envelopes are an acidic subset of the total matrix 60,000–70,000-dalton polypeptides, which range from acidic to basic on two-dimensional acrylamide gels. Moreover, a recent study by Agutter and Birchall (2) suggests significant functional differences between the lamina structure and the internal matrix structure.

Localization of Matrix-associated RNA and DNA

Previous studies of the nuclear matrix have demonstrated an association between matrix proteins and newly replicated DNA (4, 5, 6, 9). The nature of this interaction, however, has yet to be resolved. Recently, Comings (18, 20) has reported an *in vitro* DNA binding capacity for the nuclear matrix proteins. The association of 70% of the total matrix-associated DNA with isolated matricin before DNase digestion suggests that the 60,000–70,000-dalton matrix proteins may function as DNA binding proteins. Experiments are in progress to determine the DNA binding properties of matricin and the possible preferential association of newly replicated DNA with this protein structure. It is of interest that the association of newly

replicated DNA with the nuclear matrix in regenerating liver is preceded by an enhanced phosphorylation of high molecular weight secondary matrix polypeptides (3). Whether these phosphorylated secondary matrix proteins are actually involved in functions associated with the replicating DNA, however, remains to be determined.

Under certain isolation conditions, ribonucleoprotein particles containing hnRNA (hnRNP) are largely composed of a series of major core polypeptides of between 30,000 and 45,000 daltons (13, 14). As is shown in Fig. 5, polypeptides in this molecular weight range (27,000–44,000) are present in the nuclear matrix and fractionate completely into the residual RNP fraction. That the core hnRNP proteins are not the major proteins in this RNP fraction is not unexpected because electron microscope observations reveal the presence of large amounts of matricin still associated with the matrix particles (Fig. 4). The presence of substantial amounts of 60,000–70,000-dalton polypeptides in the RNP fraction (~20% of total stained protein) is consistent with these structural observations. In addition, the 2 M NaCl extraction is likely to extract a large proportion of the core hnRNP proteins (13). What remains are 2 M NaCl-resistant residual components. Whether there is enough matricin in the RNP fraction (Fig. 4) to account for the observed amounts of 60,000–70,000-dalton proteins on the acrylamide gels (see Fig. 5 and Table III), however, is unclear. In this regard, some investigators have actually reported a greater heterogeneity of polypeptides in isolated hnRNP particles (16, 24, 35, 43). These RNP particles are often isolated as polyparticles and have polypeptide profiles that bear some resemblance to the residual RNP fraction, including substantial amounts of polypeptide in the molecular weight range of 60,000–75,000. The possibility that at least some of these polypeptides are matrix proteins deserves serious attention in future investigations.

It is also interesting to consider whether matricin and RNP structures interact at specific attachment sites. Such specific interactions could help to provide an organized structural system for the intranuclear transport of RNP particles (4, 9, 52, 53). Our electron microscope observations, for example, suggest that the matrix particles may be linked into polyparticles by association with matricin (Figs. 2 and 4). An association of hnRNP particles with the nuclear matrix has also been suggested by Miller et al. (41), who demonstrated the predominant association of rapidly labeled RNA with

the nuclear matrix and the release of hnRNP particles from the nuclear matrix when prepared in the absence of protease inhibitors.

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